Conclusions: We showed that CRP levels are associated with OA-related knee pain in women. This might indicate that there is an inflammatory component present in knee OA that contributes to knee pain experienced in OA. In addition, we showed that BMI modifies the effect of CRP levels on KOA. This may indicate a difference in pathogenesis of OA in overweight women compared to women with a healthy weight. For example, mechanical load is a major contributor in overweight women, whilst in women with a healthy weight systemic inflammation might play a more prominent role.
Another explanation could be that we do not see the effect in overweight women because of confounding by co-morbidity such as cardiovascular disease or diabetes, although we tried to adjust for this in our analysis.

470 COMPARING PLACEBO RESPONSE IN NORTH AMERICAN AND EUROPEAN PATIENTS WITH KNEE OA

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Purpose: In OA trials, rates of placebo response are often 50%+. Despite this, little is known about sociodemographic or clinical predictors. We hypothesized that rates of response and predictors would be similar between patients in North America (NA) vs. Europe (EUR).
Methods: Data were drawn from 622 males and females randomized to the placebo arm of a large multinational clinical trial designed to evaluate the clinical effects of a bisphosphonate on knee OA. Patients were 40−80 years of age who reported knee pain due to OA on most days during 1 of the prior 3 months, morning stiffness lasting <30 min or knee crepitus according to the ACR criteria for knee OA. No minimum level of pain was required. Patients also had 1+ osteophytes, joint space width (JSW) of 2−4 mm in the medial tibiofemoral compartment, and a medial compartment < lateral. WOMAC pain and function scales were administered at baseline and 6 months. OARSI 2004 Responder Criteria were used to classify participants at 6 months.
Results: 552 (83%) patients completed the 6 month evaluations. Overall, patients were mostly female (63%) and white (79.2%) with a mean (±SD) age of 62.0±8.8 years, BMI of 29.8±4.6, and JSW of 3.0±0.6 mm. EUR patients were significantly ($p < 0.01$) older, more likely to be female, lighter and use less analgesic medication. EUR patients also had significantly ($p < 0.01$) higher WOMAC pain, function and patient global assessment scores at baseline.
More participants in the EUR as compared to the NA sites met the criteria for treatment response (23.5% vs. 21.0%, respectively), although the difference was not statistically significant ($p = 0.488$). A breakdown of patients meeting criteria for high response and improvement by cohort is shown below.
At baseline, placebo responders had significantly lower scores for WOMAC pain (31.6±1.5 vs. 41.6±1.1; $p < 0.001$), function (37.8±1.7 vs. 44.9±1.2; $p < 0.001$) and patient global assessment (50.2±2.2 vs. 55.4±1.1; $p = 0.032$). No statistically significant differences were found between responders and non-responders for age, gender, body mass index, baseline JSW, OARS grade, osteophytes or use of pain medication. In multivariate logistic regression models, even with adjustment for baseline sociodemographic and OA-related characteristics, baseline pain was the only consistent predictor of placebo response in both joint and separate models.
Conclusions: Using OARSI Response Criteria, more than 1 in 5 participants who were in the placebo arm met stringent criteria for treatment response. Responders were more likely to report moderate levels of pain and impairments in physical function than non-responders at baseline. Overall, there do not appear to be sociodemographic or OA-related characteristics reliably associated with placebo response.

Pain: Pathophysiology

471 QUANTITATIVE SENSORY TESTING IN OSTEOARTHRITIS OF THE KNEE

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Purpose: To assess skin sensitisation over the medial aspect of the knee in people with osteoarthritis (OA) of the knee, using quantitative sensory testing (QST) and relate this to their pain experiences
Methods: 28 patients with established medial compartment tibiofemoral joint OA were recruited to the study. They were interviewed about pain, and they completed WOMAC, HADs and MGill pain questionnaires. QST was then used to assess any area of altered sensation in the skin, test for mechanical sensitivity and pain thresholds, and assess skin thermal sensitivity.
Results: The group included 20 women and 8 men with a mean age of 62.8 years, knee pain of an averge of 5 years duration, and radiographic evidence of medial compartment knee OA. Their mean WOMAC and HADs scores were 47.8 (range 16−73) and 14.2 (6−31) respectively. 26/28 had an area of altered sensation over the medial aspect of the index knee. Sensitivity to touch was lower in that knee, but pain threshold higher than in the contralateral knee. 19/28 had thermal allodynia, severe in 4. Those with cold allodynia had higher WOMAC and HADs scores than the whole group, and were more likely to use McGill pain descriptors suggesting of neuropathic pain.
Conclusions: Sensory processing is altered in most patients with knee OA, and in some cases neuropathic pain may make an important contribution to the pain experience. This has implications for therapy.

472 SYNOVITIS INCREASES THE RISK OF KNEE PAIN IRRESPECTIVE OF STATUS OF X-RAY OA: THE MOST STUDY

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Purpose: Pain from knee OA is a key symptom in the decision to seek medical care and an important antecedent to disability. Synovitis has been hypothesized as one pathological feature that causes pain. Synovial thickening on contrast enhanced (CE) MRI has been shown to correlate well with synovitis on histology. On non CE MRIs synovial thickening cannot be assessed and on these images synovitis has been weakly and inconsistently associated with pain. We used CE MRIs, to assess synovial thickening in relation to knee pain severity among subjects in the Multicenter Osteoarthritis Study (MOST).
Methods: MOST is a NIH-funded longitudinal observational study of risk factors for knee structural changes and occurrence of pain in persons age 50−79 with or at high risk of knee OA. An unselected subset of participants who volunteered obtained 1.5 CE MRI of one knee at the 30 month clinic visit. Synovitis was scored 0−3 in 4 compartments (suprapatellar pouch, medial and lateral parapatellar recesses and infrapatellar fat pad) and 0−1 in 2 compartments (medial and lateral posterior condylar) (Rheumatol 44:1569 2005). We categorized synovitis in the whole knee as severe (>1 compartment scored 3), moderate (>2 compartments scored 2 but no 3s), mild (>4 compartments scored 1 or 1 scored 2), normal/questionable (<4 compartments scored as 1). Inter-reader kappa was 0.9 ($p < 0.001$). Subjects were asked about their knee pain severity using WOMAC. Each of the 5 items in the WOMAC pain scale is scored from 0 (no pain) to 4 (extreme pain). We used the maximum item score to define the categories of knee pain severity: no-pain (all 5 items scored 0 prior to a clinic visit), mild-pain (maximum score of any item 3). We examined the association between synovitis and pain severity using a logistic regression model adjusting for age, sex, BMI, MRI bone marrow edema, and effusions. We also examined if the effect of synovitis on pain severity was modified by radiographic OA status.

Table 1

All subjects	Synovitis			
Worst pain on any WOMAC pain question	Normal/ questionable (n = 151)	Mild (n = 233)	Moderate/severe (n = 69)	
None (n = 158)	69 (46%)	79 (34%)	10 (15%)	
Mild (n = 149)	45 (29%)	79 (34%)	25 (36%)	
Moderate/severe/extreme (n = 146)	37 (25%)	75 (32%)	34 (49%)	
Adj OR for mild pain vs no pain (95% CI)*	1.0	1.4 (0.9, 2.4)	3.1 (1.2, 8.0)	p for trend 0.02
Adj OR for moderate/ severe/extreme pain vs no pain (95% CI)*	1.0	1.9 (1.0, 3.3)	4.8 (1.8, 12.3)	p for trend 0.001
Subjects KL < 2, no PF OA	n = 132	n = 149	n = 16	
None	62 (47%)	61 (41%)	5 (31%)	
Mild/moderate/severe/extreme	70 (53%)	88 (59%)	11 (69%)	
Adj OR for pain (95% CI)*	1.0	1.4 (0.8, 2.3)	2.2 (0.6, 8.1)	p for trend 0.15

*Adjusted for age, sex, BMI and MRI bone marrow edema and effusions

Results: Of 535 subjects with CE MRI, 453 subjects had complete data on synovitis and WOMAC pain. Mean age was 59.2±7.2 years, mean BMI 29.7±5.0, and 48% were women. Moderate/severe synovitis was uncommon (15% of knees) but of knees with at least moderate pain, 49% had moderate/severe synovitis with 4.8 times increased odds of knee pain compared to those without synovitis. Other WOMAC definitions of pain severity yielded similar results. Among knees without ROA (n = 297), while few had moderate/severe synovitis, synovitis itself was still associated with increased prevalence of pain.
Conclusions: Moderate/severe synovitis has a strong relation with knee pain severity, an association detected more clearly with CE MRI than suggested by prior studies using nonCE MRI measures of synovitis.

Proteomics & Metabolomics

473 DIFFERENTIAL PROTEOME OF ARTICULAR CHONDROCYTES FROM PATIENTS WITH OSTEOARTHRITIS

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Purpose: Cartilage damage is a major problem in osteoarthritis (OA). To gain insight into the pathogenesis of OA, we have analyzed the differential proteome of articular chondrocytes from these patients.
Methods: Protein extracts were prepared from cultured chondrocytes from 6 patients with end-stage OA and 6 normal donors without special radiographic signs of OA. Samples were then analyzed by 2D gels using the DIGE approach. Gel image analysis was performed using the DeCyder Differential Analysis Software Release 6.5 and statistical module EDA 1.0 (GE Healthcare). Protein spots corresponding to statistically significant expression differences were excised from the gels and submitted to tryptic digestion, and the resulting peptides were mass analyzed using an Ultraflex MALDI-TOF/TOF mass spectrometer (Bruker-Daltonics). Protein identification was achieved through database searching with MALDI MS and MS/MS data.
Results: Significant differential expression was observed for 27 proteins, with 14 underexpressed and 13 overexpressed chondrocyte OA proteins. Of special interest was the identification of destrin, cofilins, gelsolin, annexin A2, glycolytic enzymes, chaperones, cathepsin D, proteasome beta 9 subunit isoform 2 proprotein and proteasome activator hPA28.
Conclusions: The altered expression of these proteins is consistent with events such as cytoskeleton binding protein disruption, apoptosis, and glycolysis, demonstrating the ability of the 2D-DIGE/MS platform to identify proteins with altered expression in chondrocytes from patients with end-stage OA. The identification of these proteins may open new lines of research for this disease.

474 A ROBUST METHOD FOR PROTEOMIC CHARACTERIZATION OF MOUSE CARTILAGE USING SOLUBILITY-BASED FRACTIONATION AND TWO-DIMENSIONAL ELECTROPHORESIS

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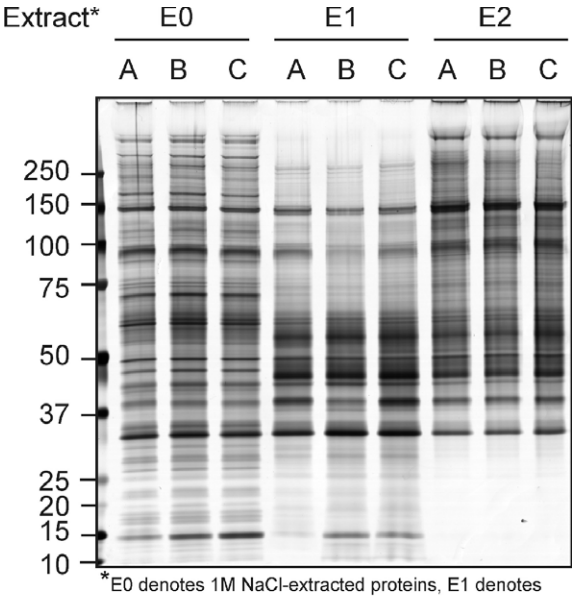
Purpose: There is increasing interest in proteomic techniques for global profiling of normal and pathological cartilage samples, to elucidate underlying disease mechanisms identify novel biomarkers. Identification of protein expression differences using two-dimensional electrophoresis (2-DE) and liquid chromatographic (LC)-based proteomics depends critically on reproducibility throughout sample preparation and analysis. This applies particularly where sample fractionation is used to remove high abundance or interfering components to facilitate deeper mining of the proteome. Here we have developed and validated a procedure for solubility-based cartilage sample fractionation and reproducible resolution by 2-DE.
Methods: Triplicate independent *sequential* extractions were performed on mouse femoral head cartilage. Pulverized explants were digested with chondroitinase ABC then extracted in Tris acetate containing 1 M NaCl for 24 hrs (E0 fraction). The NaCl-insoluble fraction was then further extracted for 18 hrs in sodium acetate containing 4 M GuHCl (E1 fraction). Finally, to prepare samples for 2-DE, high molecular weight components that prohibit isoelectric focusing (hyaluronan and aggrecan) were removed by 100 kDa cut-off centrifugal filtration (E2 fraction). Triplicate E0, E1 and E2 fractions were first profiled by SDS-PAGE. E0 and E1 were then resolved by 2-DE in triplicate to compensate for variation in 2-DE and silver staining and the 18 gel images were analyzed in ImageMaster. The E0 and E1 extracts were characterized by identification of protein spots by tandem mass spectrometry (MS).
Results: The 1-D profiles of E0, E1 and E2 fractions were highly consistent between extractions (see figure). Centrifugal filtration caused partitioning of some proteins <100 kDa into the E2 fraction but this was also consistent between samples. E0 and E1 fractions produced distinct protein 2-DE spot patterns, with greater complexity in E0. Automated spot analysis reported 70% spot matching in E0 gel triplicates and 75% matching in E1 gel triplicates, representing approximately 600 and 500 matched spots, respectively. E0-specific spots were mostly cellular proteins, e.g. BiP, triosephosphate isomerase and gelsolin, whereas E1-specific spots were abundant matrix proteins, e.g. collagen VI, matrilins 1 and 3, and lactadherin. Interestingly, some specific proteins such as link protein and beta-actin partitioned almost equally between E0 and E1 extracts. MS results were validated by immunoblotting.



*E0 denotes 1M NaCl-extracted proteins, E1 denotes 4M GuHCl-extracted proteins and E2 denotes proteins removed by 100 kDa cut-off centrifugal filtration

Conclusions: This study has, using the minimal amounts of tissue available from mouse tissue, established a new approach to 2-DE based analysis of cartilage extracts. This method can be used to enrich one or both protein fractions for deeper mining of the cartilage proteome and to investigate cellular mechanisms and matrix components involved in developmental and degenerative cartilage disease.

475 PROTEOMIC CHARACTERIZATION OF MOUSE CARTILAGE DEGRADATION *IN VITRO*

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Purpose: To develop proteomics to analyze mouse cartilage degradation and correlate transcriptional and translational responses to catabolic stimuli.
Methods: Proteomic techniques were used to analyze catabolism in mouse femoral head cartilage. Using specific methods to prepare cartilage extracts and conditioned media for two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) and subsequent tandem mass spectrometry (MS), we identified novel proteins and fragments released into the media of control, interleukin-1α (Il-1) and all-*trans*-retinoic acid (RetA)-treated explants. Fluorescent difference gel electrophoresis (2-D DIGE) was used to quantify protein expression changes. We also measured changes in mRNA expression to distinguish transcriptional and post-translational regulation of released proteins.
Results: We identified 20 differentially-abundant proteins in media from control and treated explants, including fragments of thrombospondin-1